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Steric Effects Direct the Binding of Porphyrins to Quadruplex DNA

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Experimental

(5,10,15,20-tetrakis-(N-methylpyridinium-4-yl)porphyrinato)copper(II) (Cu(T4)), (5,15-dimethyl-10,20-di(N-methylpyridinium-4-yl)porphyrinato)copper(II) (Cu(tMe_2D4)), and (5,15-dimethyl-10,20-di(N-methylpyridinium-4-yl)porphyrinato)zinc(II) (Zn(tMe_2D4)) were prepared as the chloride salts according to previously published methods[S1] and the purity of each was confirmed by absorption and emission spectroscopy. All DNA oligonucleotides were purchased from Integrated DNA Technologies and were used without further purification. Dichlorodimethylsilane, Trizma HCl, and Trizma base were purchased from Sigma-Aldrich. KCl was purchased from Mallinckrodt Chemicals. Reverse osmosis water was passed through a Barnstead Bantam water purification system with a mixed-bed cartridge before use. All glassware used for storage or measurements of cationic porphyrin solutions was silanized to prevent adsorption. Tris buffer (pH=7.5, μ=0.05 M) was prepared from Trizma HCl and Trizma base. This buffer was used to make a 1 M solution of KCl used for stabilization of the DNA quadruplex structure. The porphyrin of interest was dissolved in Tris buffer to create a stock solution, which was stored in the dark at 4^{o}C. Molar absorptivity was used to determine the concentration of stock and experimental solutions. The stock solution was diluted with Tris buffer to afford a 2 μM porphyrin solution for studies. To the initial porphyrin solution 75 μL of 1 M KCl in Tris buffer solution was also added, to afford a 50 mM KCl starting solution. The final KCl concentration is never less than approximately 35 mM. The concentration of porphyrin was held constant throughout all experiments. Stock solutions of DNA oligos were dissolved in 1 mL of Tris buffer. Concentration was determined using the molar absorptivity reported from the manufacturer. It was assumed that the molar absorptivity of quadruplex is four times that of the single stranded oligo. Aliquots of the DNA oligo stock solution of interest were added to the porphyrin starting solution to give the desired concentration. All spectra were measured at room temperature after mixing. Emission spectra were corrected for absorbance differences at the exciting wavelength due to the lack of an isosbestic point and for detector imperfections using the manufacturers correction factors. CD spectra were baseline corrected to free buffer solution and zeroed at 510 nm.

Table S1. Molar absorptivity at λ_{max} for each porphyrin investigated.

Porphyrin (λ_{max})	$\varepsilon, M^{-1}s^{-1}$
Cu(T4) (425 nm)	231,000
Cu(tMe_2D4) (420 nm)	170,000
Zn(tMe_2D4) (430 nm)	150,000

Table S2. DNA oligonucleotide molar absorptivity values.

DNA	$\varepsilon, M^{-1}s^{-1}$
TG_4T	57,800
$T_2G_4T_2$	74,000
$T_3G_4T_3$	90,200
$T_4G_4T_4$	106,400
T_2G_4	57,400
T_4G_4	73,600

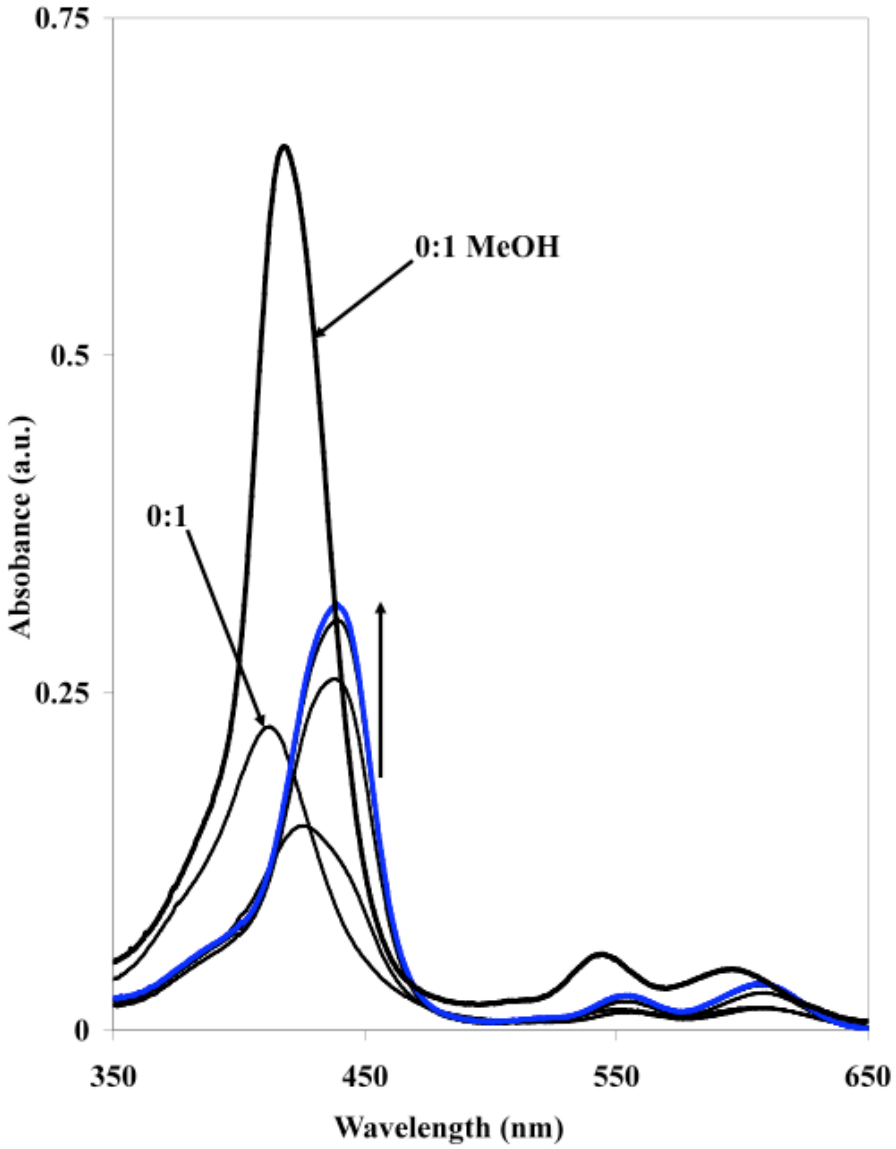

Figure S1. Absorbance spectra of Cu(tMe_2D4) during a $T_4G_4T_4$ titration. Thick trace is in methanol at 2 μM porphyrin, 0:1 is in buffer at the same concentration also without DNA. Blue trace represents the limiting spectrum with a nominal DNA concentration of 16 μM strand and 2 μM Cu(tMe_2D4). Other traces are at 2, 4, and 8 μM DNA. The absorbance of porphyrin increases from 2 to 16 μM strand concentration due to the loss of aggregates and/or exciton interactions that occur at high loading of ligand.[S1]

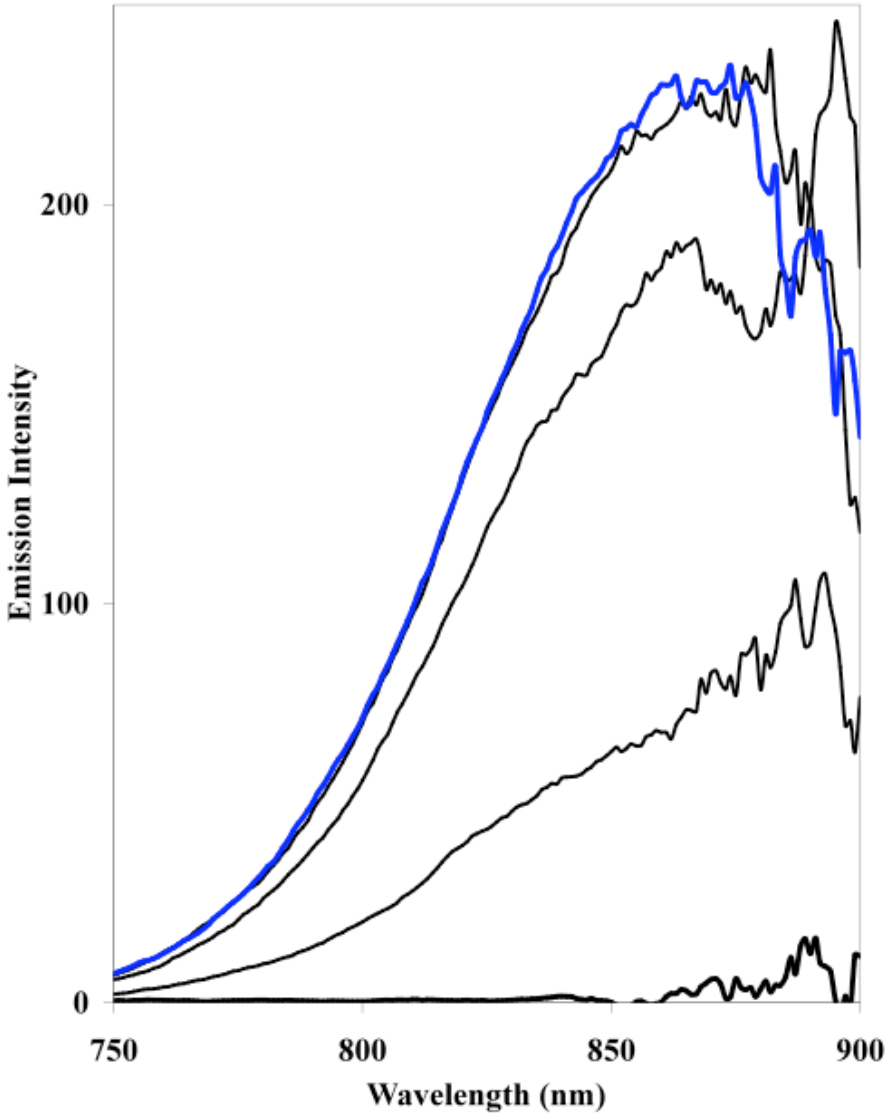

Figure S2. Emission spectra of Cu(tMe_2D4) during a $T_4G_4T_4$ titration. Thick black is at 2 μM concentration in the absence of DNA and blue represents the limiting spectrum with a nominal DNA concentration of 16 μM strand and 2 μM Cu(tMe_2D4). Other traces are at 2, 4, and 8 μM DNA in order of increasing emission intensity. All data have been corrected for hypochromicity and the wavelength dependent instrumental response.

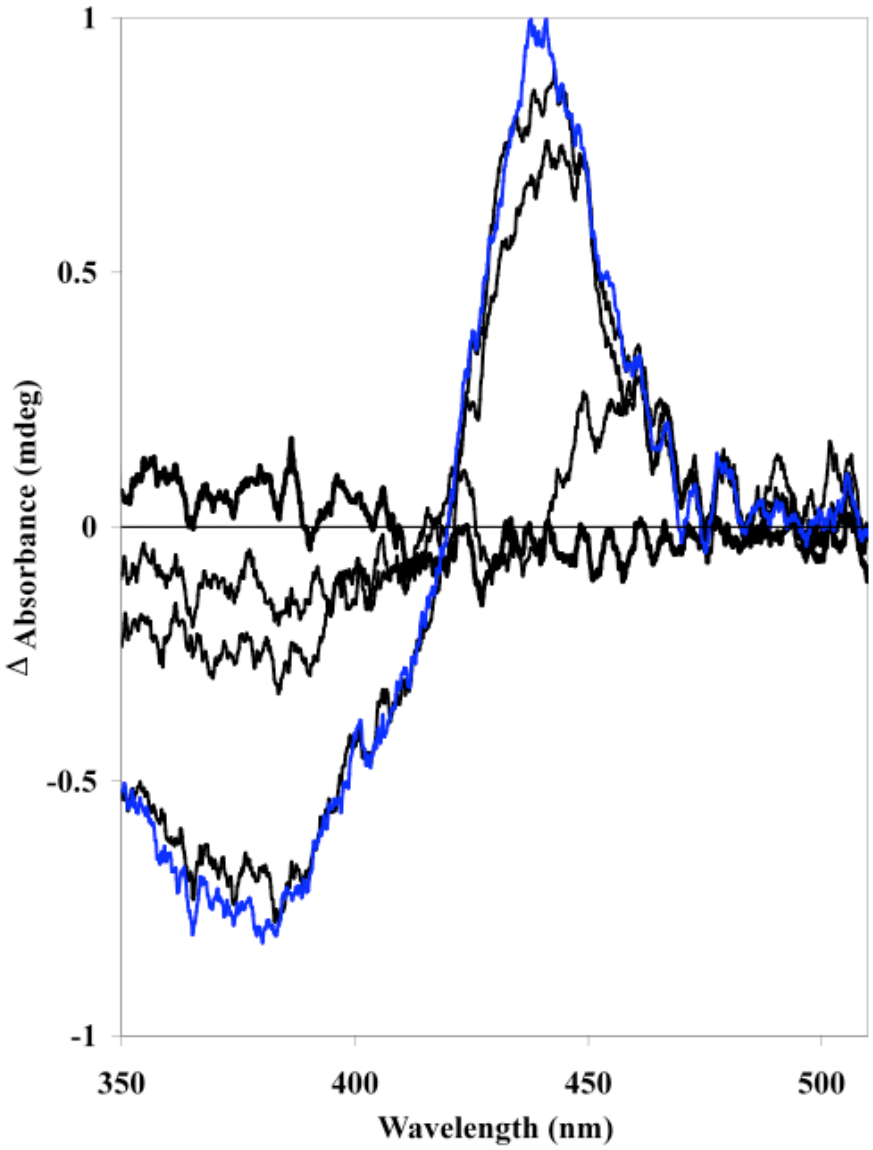

Figure S3. Induced CD spectra of Cu(tMe_2D4) during a $T_4G_4T_4$ titration. Thick black is in the absence of DNA and blue represents the limiting spectrum with a nominal DNA concentration of 16 μM strand and 2 μM Cu(tMe_2D4). Other traces are at 2, 4, and 8 μM DNA in order of increasing amplitude.

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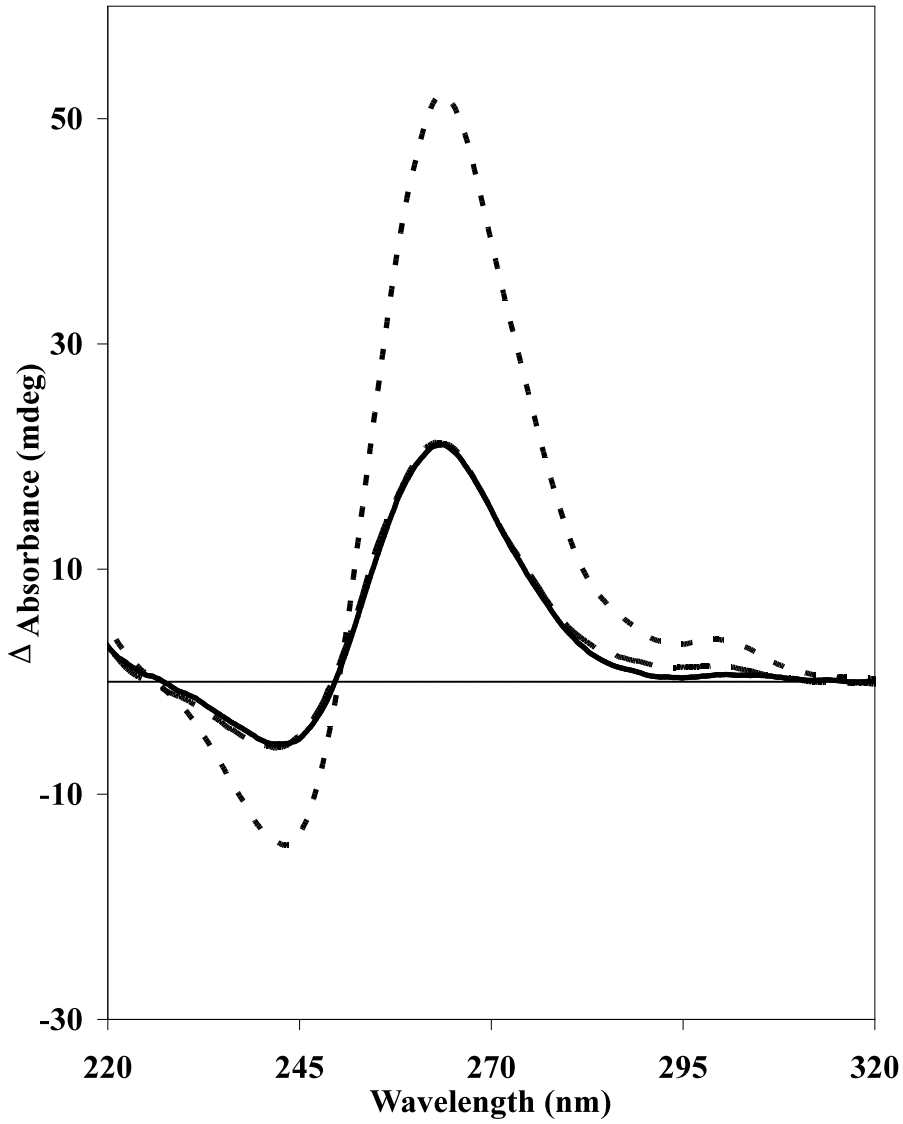

Figure S4. CD signal of TG_4T in the UV region at 16 μM strand concentration (solid) or 8 μM strand concentration with 2 μM Cu(tMe_2D4) (small dashes) and Cu(T4) (long dashes).

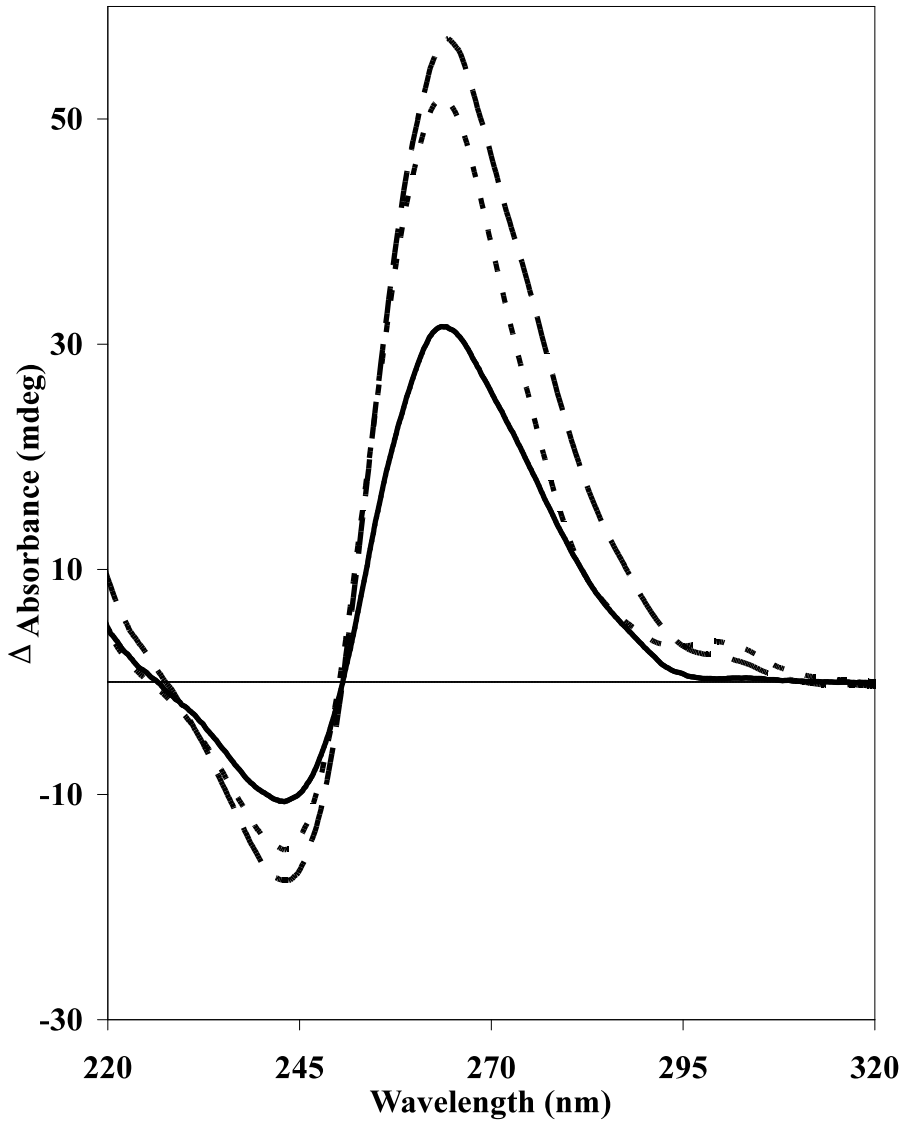

Figure S5. CD signal of $T_2G_4T_2$ in the UV region at 16 μM strand concentration (solid) or 8 μM strand concentration with 2 μM Cu(tMe_2D4) (small dashes) and Cu(T4) (long dashes).

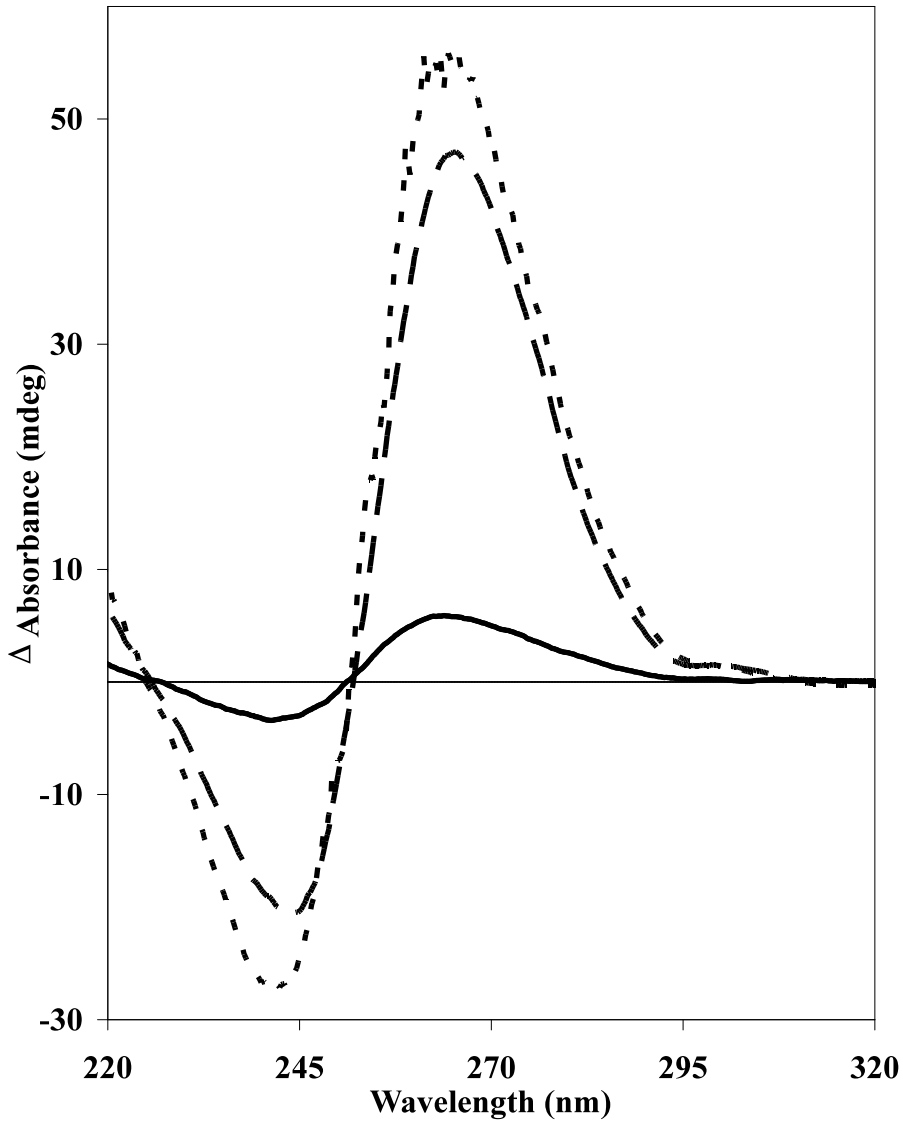

Figure S6. CD signal of T_4G_4 in the UV region at 16 μM strand concentration (solid) or 8 μM strand concentration with 2 μM Cu(tMe_2D4) (small dashes) and Cu(T4) (long dashes).

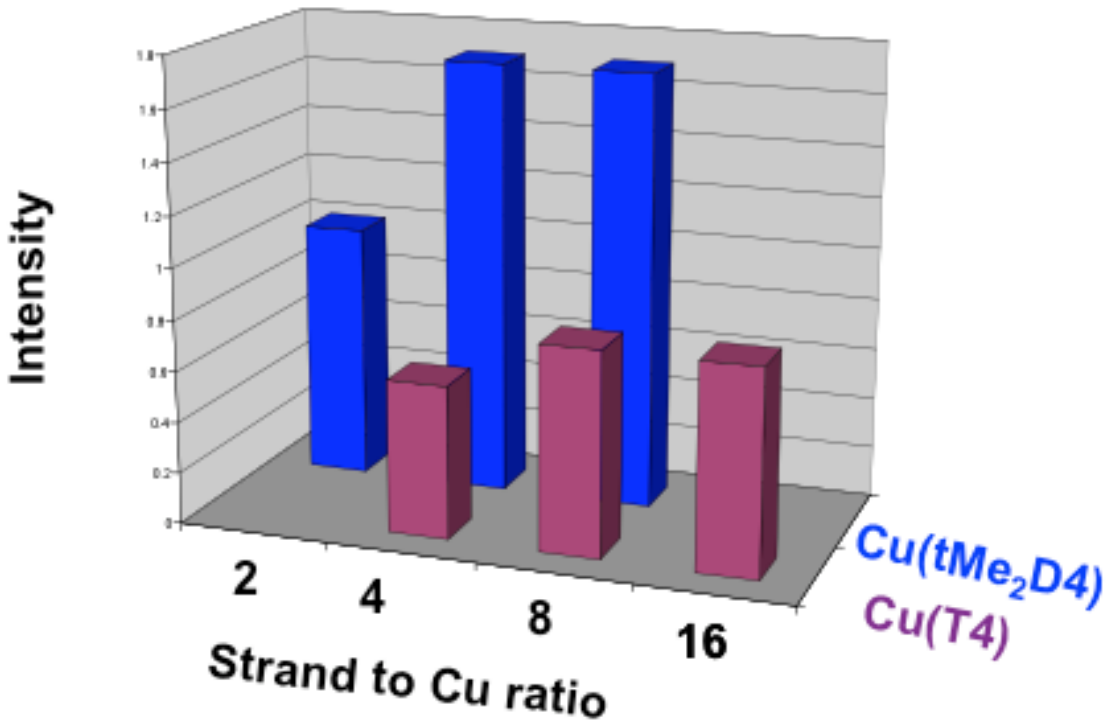

Figure S7. Emission intensity of 2 μM Cu(tMe$_2$D4) and Cu(T4) with T_4G_4 at various strand DNA concentrations.

References

S1. Shelton, A. H.; Rodger, A.; McMillin, D. R. *Biochemistry* **2007**, *46*, 9143-9154.